Prospective translational study investigating molecular PrEdictors of resistance to first-line PazopanIb in Metastatic reNal CEll Carcinoma (PIPELINE Study)

Prospective translational study **I**nvestigating molecular Pr**E**dictors of resistance to first-**L**ine pazopan**I**b in metastatic re**N**al c**E**ll carcinoma (**PIPELINE** Study)

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Abstract

Background: tyrosine kinase inhibitors have significantly improved the outcomes in metastatic renal cell carcinoma (mRCC) patients (pts). Despite initial clinical benefit, resistance to anti-angiogenic therapies develops through the activation of alternative angiogenic pathways. Plasma levels of circulating angiogenic factors (CAFs) were mesured in pts with mRCC treated with pazopanib to identify predictive biomarkers of resistance. **Methods:** PTS with mRCC treated with pazopanib in first-line at Istituto Nazionale Tumori of Milan between July 2015 and February 2017 were enrolled in this prospective trial. Levels of 7 CAFs of interest including interleukin-6 (IL-6), interleukin-8 (IL-8), stromal cell-derived factor 1 (SDF-1), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), Osteopontin and E-selectin, quantified by immunometric technology, were obtained before treatment and every 4 weeks until disease progression (PD) defined by RECIST Criteria 1.1. Wilcoxon test for paired samples was used to compare CAFs levels at baseline (B) and PD. **Results:** Overall, 25 pts were included in the final dataset. Median follow up was 31.9 months. As best response, 12 patients presented partial response (48%), while 9 presented a stable disease and 4 a PD. At time of analysis, 6 patients (24%) were still on treatment, 15 (60%) discontinued treatment for PD and 4 (16%) stopped pazopanib due to toxicity. Median progression-free survival was 14.8 months. Paired plasma samples from the 15 pts with PD were analyzed. Overall, median plasma levels of SDF-1 and VEGF resulted significantly higher at PD compared to B [SDF-1: B 574,67 pg/mL (range 200,8-2.018,39) vs PD 1328,03 pg/mL (range 472,55-2.126,96) p=0,011; VEGF-A: B 45,10 pg/mL (range 6,16-256,14) vs PD 62,4 pg/mL (range

39,42-186,74) p=0,011]. Conversely, median levels of E-selectin were significantly lower at PD compared to B [B 23.882,51 pg/mL (range 11.016,44-56.948,61) vs PD 20.588,30 pg/mL (range 10.991,75-38.415,71) p=0,017]. None of the remaining CAFs evaluated showed a significant variation between B and PD. Additionally, patients with lower baseline levels of HGF showed longer PFS and OS, while lower baseline levels of IL-8 showed longer OS compared to patients with the higher corresponding CAF. **Conclusions**: Low baseline levels of IL-6, IL-8, HGF and Osteopontin were associated to tumor response to pazopanib while higher plasma levels of SDF-1 and VEGF-A were associated with PD during first-line pazopanib than at B.Thus, levels of selected CAFs during treatment with pazopanib may represent potential candidates to predict resistance and PD to therapy. These findings warrant further investigation in larger trials.

Key words: renal cell carcinoma, circulating angiogenic factors, TKI, pazopanib,targeted therapies, circulating biomarkers

Introduction

Renal cell carcinoma (RCC) is a highly vascular tumor, arising from epithelial elements within the proximal tubules of nephrons. Overexpression of vascular endothelial growth factor (VEGF), often due to the alteration of the *von Hippel-Lindau* (*VHL*) gene, stimulating tumor growth and angiogenesis, plays an important role in RCC pathogenesis (1). Several agents that target the VEGF pathway in different ways, i.e. blocking the pathways that regulate hypoxia-inducible factors (HIFα) levels, directly inhibiting the function of VEGF, or interrupting the signaling cascade downstream the VEGF receptor via tyrosine kinase inhibitors (TKIs), are approved for the treatment of RCC (2) (3) (4). In the last decades, the anti-vascular TKIs significantly improved outcomes in metastatic renal cell carcinoma (mRCC) patients (3) (4). Among TKIs, pazopanib is a potent multitarget inhibitor of VEGF receptors (VEGFR) 1, 2, and 3, PDGF receptors α and ß and stem cell factor receptor (c-Kit) (5), with a higher binding affinity in vitro for VEGFR-2 compared to sunitinib (6). Pazopanib showed to improve progression-free survival (PFS) and overall survival (OS) in mRCC with a comparable efficacy to sunitinib (7) (8) (9) (10). However, about 20% of patients treated with TKIs derive no benefit from the treatment due to primary resistance, while the remaining proportion of patients could experience disease progression due to secondary resistance after an initial response to the treatment, after a median time of one year (11) (12).

Thus, understanding the mechanisms underlying primary and secondary resistance to TKIs is fundamental to identify predictive biomarkers to guide the future treatment choice and to develop new targeted agents with the aim to improve patients'clinical outcomes.

Several studies reported that the activation of alternative angiogenic pathways in both tumor cells and stroma could contribute to develop resistance to treatment (13) (14). Despite a successful inhibition, in fact, the activation of alternative ligands/receptors that sustain the signaling of key downstream pathways could lead to escape the pharmacological inhibition of the VEGF/VEGFR-axis (15). Different mechanisms involved in resistance are reported in literature, such as MET proto-oncogene receptor tyrosine kinase (MET)/hepatocyte growth factor (HGF) pathway activation and IL-8 (10) (16). Preclinical studies detected higher HGF levels in the stromal compartment of resistant tumor and an overexpression of its receptor c-Met on the endothelial tumor cell surface (17). Concerning IL-8, it is a chemokine member of the CXC family implicated in tumor growth and angiogenesis. An increased serum level and tissue overexpression of IL-8 was in fact documented in primary resistant mRCC models (18) (19) (20). Changes in plasma cytokines and circulating angiogenic factors (CAFs), including IL-8, IL-6, SDF1, HGF, FGF, VEGF, and Osteopontin may therefore provide evidence for the biologic activity of pazopanib and could retain the potential to be used as serum biomarkers able to predict drug response and/or resistance. The use of CAFs as potential surrogate biomarkers has been examined in mRCC, but unfortunately no prospective trials are ongoing to evaluate them (21).

PIPELINE trial, was designed to assess prospectively the plasma levels of CAFs in patients with mRCC treated with pazopanib as first line therapy, .

Here, we report the results from translational analyses on change in circulating biomarkers (IL-6, SDF1, IL-8, Osteopontin, VEGF, HGF and FGF) between blood samples taken at baseline (before starting treatment) and at the time of PD as per RECIST 1.1 in order to better understand changes as resistance develops. At time of manuscript writing, analysis on tissue samples were ongoing.

2. Material and Methods

2.1 Study design

This prospective single-centre translational research study, the PIPELINE study, conducted at Fondazione IRCCS Istituto Nazionale dei Tumori of Milan Italy, was designed to evaluate biomarkers associated with drug resistance to TKI in a cohort of mRCC patients suitable to receive pazopanib as first line treatment. Pazopanib was given 800 mg orally daily (cycles of 28 days) until disease progression (PD) or unacceptable toxicity.

Our study included the collection of blood and tumor tissue samples. Blood samples were obtained at baseline, during treatment and at time of PD. Tumor tissue samples were obtained for all patients by collecting pre-existing archival tumor specimen if available, or by a fresh biopsy before treatment and, optionally, upon progression if safe and technically feasible.

Tumor restaging was performed every 12 weeks +/- 7 days, as per clinical practice. Patients were monitored until PD, withdrawal from the study, intolerable toxicity or study completion. All the patients provided written informed consent before undergoing any trial procedures. All the procedures set out in this study were consistent to Good Clinical Practice Guidelines and Declaration of Helsinki.

2.2 Patients

Eligible patients were adults ≥18 years old with histological confirmed diagnosis of RCC displaying a clear cell component and/or sarcomatoid features not previously treated with any systemic treatment, including agents in the adjuvant setting. Patients should have evidence of advanced or metastatic disease with measurable lesions according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Patients were required to have Eastern Cooperative Oncology Group (ECOG) performance status of 0 and 1, adequate bone marrow, liver, renal and pancreatic function. Mandatory was the availability of either an archival or newly collected formalin-fixed, paraffin-embedded (FFPE) tumor tissue sample.

Patients were excluded if they had diagnosis of concomitant cardiac disorders including uncontrolled hypertension, clinically significant gastrointestinal abnormalities that could increase the risk for gastrointestinal bleeding, history of cerebrovascular accident including transitory ischemic attack (TIA), pulmonary embolism or untreated deep venous thrombosis (DVT) within the past 6 months, major surgery or trauma within 28 days prior to first dose of pazopanib and/or presence of any non-healing wound, fracture, or ulcer, known endobronchial lesions and/or lesions infiltrating major pulmonary vessels that increase the risk of pulmonary hemorrhage.

2.3 Procedures

Blood collection

Blood samples were collected for all eligible patients at baseline, every 4 weeks during pazopanib treatment and at the time of PD defined by RECIST 1.1. Whole blood (10 ml) was collected in K2EDTA Vacutainer tubes and plasma was separated by centrifugation 3000 rpm 4°C for 15 min. Plasma levels of 7 circulating angiogenic factors (CAFs) of interest, including IL-6, IL-8, VEGF-A, HGF, Osteopontin and E-selectin, were quantified by Luminex® technology, (Luminex Human Magnetic Assay 6-Plex LXSAHM-06 HGF, IL-6, IL-8/CXCL8, Osteopontin, E-Selectin, VEGF-A) as per standard protocol. Luminex® technology Assays utilize color-coded superparamagnetic

beads coated with analyte-specific antibodies. Beads recognizing different target analytes are mixed together and incubated with the sample. Captured analytes were subsequently detected using a cocktail of biotinylated detection antibodies and a streptavidin-phycoerythrin conjugate. Conversely, plasma level of SDF-1 was determined using the quantitative sandwich enzyme immunoassays (ELISA) assay kits, Quantikine® by R&D Systems® (Human CXCL12/SDF-1 alpha Quantikine) as per standard protocol.

Furthermore, at baseline and every 12 weeks during pazopanib treatment and at the time of PD whole blood (30 ml) was collected for peripheral blood mononuclear cells (PBMC) separation and storage in liquid nitrogen for subsequent analyses, together with corresponding plasma samples.

All patients provided written informed consent before undergoing any trial procedures.

2.4 Objectives

Primary objectives of the study were: to identify molecular predictive biomarkers of resistance to first line treatment with pazopanib in mRCC by using next-generation sequencing (NGS) methods; Overall Response Rate (ORR) defined as the rate of complete response (CR) plus partial response (PR) as per RECIST 1.1 to pazopanib.

Secondary objectives were: to collect blood samples from mRCC patients treated with pazopanib in first line to identify circulating predictive biomarkers of resistance/response to TKI; to compare change in promising circulating biomarkers (including SDF1, IL-6, IL-8, Osteopontin, VEGF, HGF and E-selectin) between blood samples taken at baseline and at the time of PD as per RECIST 1.1 in order to better understand changes in the tumor and in the levels of CAFs when resistance develops; to collect prospective and retrospective demographic, clinical and pathological data to correlate change in biomarkers to clinical outcomes; to perform subgroup analyses comparing the tissue and blood biomarkers identified in patients who developed secondary resistance with those biomarkers identified in patients who have primary resistance; to assess the modulating effect of pazopanib treatment on immune cell profile (in terms of frequency and function of different lymphocytes and myeloid cell populations) in peripheral blood and tumor biopsies if available. Tumor response was performed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.
At time of manuscript writing, analysis on tissue samples and immune cells profile were ongoing.

2.5 Statistical analysis

For within-patient comparison of candidate genes/biomarkers of tissue samples taken at baseline ad upon PD, we used a Mc Nemar test. To compare previously defined promising circulating predictive biomarkers for pazopanib treatment between blood samples taken at baseline and at the time of PD we used a Mc Nemar test for dichotomous outcome and Wilcoxon test for continuous data. Tables of frequencies and percentages were calculated for discrete variables. Circulating variables were analyzed by statistical descriptive procedures. Changes in CAFs were observed in terms of different expression levels in blood Patients were dichotomize into "low" and "high" cytokine and circulating angiogenic factors (CAFs) subgroups by cutoff points defined by the respective median CAF value as consistent with literature (21). The Kaplan-Meier method was used for survival analyses to estimate PFS and OS. We used the Log-Rank test to compare PFS and OS between the low and the high CAF subgroups. Correlation with ORR were performed by using Fisher exact test, statistical significance threshold was set to a canonical two-tailed 0.05 value. Given the exploratory nature of the analysis, no formal correction for multiple testing was applied.

Results

Patients

A number of 27 patients eligible for a first line treatment with pazopanib 800 mg daily for untreated mRCC were prospectively enrolled at Istituto Nazionale Tumori of Milan between July 2015 and February 2017. All patients, except for one, were caucasian and all had a confirmed clear cells histology. Among all patients enrolled, 25 patients were included in the final data set since 2 patients resulted screening failure for consent withdrawal. Median age was 65 years old (range 54 - 73). At the data cutoff date for PFS of May 5th 2019, 6 patients continued to receive the study treatment (**Figure 1**). The most common reason for discontinuing treatment was radiological PD. Median follow-up was 31.9 months. A pre-existing archival tumor specimen was available for all patients receiving study treatment. Upon progression, an optional biopsy was obtained for 3 patients. Baseline characteristics are summarized in **Table 1**.

Tumor response

Among 25 patients receiving pazopanib, partial response was observed in 12 patients, for a response rate of 48%, while 9 presented a stable disease and 4 a PD as best response. At time of analysis, 6 patients (24%) were still on treatment, 15 patients (60%) experienced a radiological PD and 4 (16%) stopped pazopanib due to toxicity. Median progression-free survival was 14.8 months (**Suppl. Fig. 1a**). Median overall survival (OS) was not reached (**Suppl. Fig. 1b**).

Blood collections

Blood samples were processed for all patients for the determination of changes in candidate biomarkers that may potentially correlate with first-line pazopanib therapy resistance.

Patients were dichotomized into two groups according to the baseline level of CAFs: "low" (≤ cutoff value) and "high" (> cutoff value) subgroups by a cutoff point chosen considering the baseline median value of each factors, as reported in literature (21). Correlation with ORR in high versus low subgroups were performed and are showed in **Table 2 - 3**. Overall, low baseline levels of IL-6, IL-8, HGF and Osteopontin showed to be significantly associated to objective response to treatment with pazopanib. However, we identified no significant association with ORR for baseline levels of SDF1, VEGF and E-selectin.

Among the 15 patients experiencing a radiological PD, changes in CAF's plasma level between blood samples taken at baseline (B) and at the time of PD were compared. Overall, median plasma levels of SDF-1 and VEGF-A resulted significantly higher at PD compared to baseline. Conversely, E-selectin was significantly lower at PD compared to basal (**Figure 2**). None of the remaining CAFs evaluated showed a significant modification between baseline and PD. Change in CAFs level are summarized in **Table 4** and illustrated in **Figure 3**.

Additionally, patients with lower IL-8 levels at baseline showed a significantly longer OS (p 0.04) compared to patients with higher levels of IL-8 at baseline (**Figure 4)**. Moreover, lower HGF levels at baseline showed longer PFS (p 0,0021) and OS (p 0.0226) compared to basal higher levels (**Figure 5**). However, we identified no significant differences in PFS and OS for other CAFs (**Suppl. Fig. 2 – 3**).

Discussion

The purpose of our study was to explore potential biomarkers of resistance or response to a first line treatment with pazopanib in mRCC patients through the assessment of plasma levels of a set of CAFs by using a commercially available kit that showed to be cost effective and easily reproducible. The assessed CAFs were IL-6, IL-8, SDF-1, VEGF, HGF, Osteopontin and E-selectin. They all are components of the angiogenesis system and molecular factors modulated by the activation of alternative anti-angiogenic pathways (14) (21) (22) (23) (24). There is strong evidence, in fact, that angiogenesis plays an important role in RCC pathogenesis and it is reported that the activation of alternative anti-angiogenic pathways could contribute in determining resistance to anti-angiogenic treatment with TKIs (13). As reported by Pal et al, RCC biology

changes during therapy and between treatment lines, with a multitude of genomic alterations arising as a consequence of selective pressure from therapy (25). For example, *VHL* tumor suppressor gene is frequently mutated in RCC and its alterations are reported to increase during disease course. These findings suggest that angiogenesis remain a key mechanism in determining resistance to TKI. In addition, recent reports suggested that *VHL* and p53 act in synergy in the regulation of cell proliferation and apoptosis. Furthermore, the regulatory role of *VHL* is dependent on the activation p53, providing a plausible explanation for VEGF directed therapy (26).

Current blood biomarkers may provide prognostic information but they are not known to be predictive. Serum levels of immunomodulatory factors including, IL-6, IL-8, HGF and Osteopontin were reported to be associated with prognosis in different studies (21) (27) (28) (29). Tran et al. showed that higher baseline IL-8, HGF and Osteopontin levels were associated with a shorter PFS in patients treated with pazopanib in first line. On the other hand, in the placebo group, high concentrations of IL-6 and IL-8 were all associated with shorter PFS (21). We also assessed whether CAFs added prognostic information and, in fact, consistent with literature, our results confirmed that patients with lower baseline levels of HGF showed longer PFS and OS, while lower baseline levels of IL-8 showed longer OS compared to patients with the higher corresponding CAF.

Thus, CAF's profile could provide prognostic information and identify potential predictive biomarkers of benefit from treatment with anti-angiogenic agents.

In our analysis, we defined two distinct and equally sized groups on the basis of basal value of CAFs that were dichotomized in "high" versus "low" considering the baseline median value of each factor. Among all CAFs, only IL-6, IL-8, HGF and Osteopontin showed to be significantly associated to objective response; i.e. patients with "low" levels of the marker had a statistically significant higher proportion of objective response to treatment. Thus, pre-treatment plasma level of these markers could be useful to predict response to pazopanib.

As reported in literature, plasma levels of proangiogenic molecules, SDF-1 and VEGF, seem to provide prognostic information. In particular, basal high levels of VEGF have been associated with worse outcome, to confirm the fact that VEGF expression levels are involved in the development and progression of renal parenchymal tumors (30) (31) (32). Moreover, both VEGF and SDF-1 were found to increase on sunitinib and correlate with outcome, reflecting severe sunitinib-induced hypoxia in the tumor (33) (34). It is now clear that antiangiogenic treatment efficiency depends on the hypoxic status of the tumor and the degree of hypoxia induced by antiangiogenic drugs, therefore this mechanism could explain the occurrence of resistance to therapy (35) (36).

In addition, SDF-1, by binding its receptor CXCR4, promotes tumor proliferation, inhibits apoptosis and enhance tumor associated angiogenesis (37) (38) working synergistically with VEGF. Our analysis showed that plasma levels of SDF-1 and VEGF resulted significantly higher at progression compared to baseline, similarly to what observed in sunitinib-treated patients (39) (40). These results suggest that even during pazopanib therapy these circulating factors may participate in mechanisms of resistance and may be a potential therapeutic target.

Conversely, we found that E-selectin was significantly lower at PD compared to baseline. Analysis of the literature highlights that E-selectin is involved in adhesion between RCC and endothelial cells and inflammatory cytokine production. Moreover, an excessive production of circulating E-selectin has an inhibitory effect on developing metastasis (41) (21). Thus, plasma level of SDF-1, VEGF and E-selectin could be used not only to provide prognostic information but also to potentially predict outcomes in patients treated with pazopanib.

To date, immunotherapy is significantly changing the frontline treatment landscape for patients with mRCC, indeed the combination of ipilimumab and nivolumab has been approved in treatment-naïve patients with intermediate- or poor-risk disease (42) (43) (44) (45). Notwithstanding this, some patients might still benefit from monotherapy with TKI as first line treatment. As recently showed by the Checkmate 214 trial, anti-angiogenic drugs remain of interest for patients with favorable risk mRCC. Thus, the introduction of biomarkers of response or resistance in the clinical practice has the potential to considerably improve the attempts to individualize patient prognostication and treatment strategies. The set of CAFs that we used in our analysis, for example, may be of value as biomarkers of the angiogenic processes and the pharmacological and clinical activity of anti VEGF-driven therapy in RCC.

Strengths of our study are the prospectively collected data and the fact that we analyzed samples of patients that were naïve to pazopanib treatment, allowing the assessment of correlation between baseline biomarkers, clinical outcome, and primary tumor response. On the other hand, the limitation of our analysis is the small sample size that did not allowed to perform a multivariate analysis and hampers the clinical and statistical significance of the results obtained; moreover, in the current series, tissue-based genomic profiling data and immune-cells profile results were not available yet.

Therefore, further research and larger studies are warranted in order to confirm the predictive value of the biomarkers explored.

Conclusion

Our analysis showed that low baseline levels of IL-6, IL-8, HGF and Osteopontin showed to be significantly associated to tumor response to pazopanib. Moreover, higher plasma levels of SDF-1

and VEGF-A were significantly associated with disease progression during first-line pazopanib. These results suggest the activation of an alternative angiogenic pathway as a mechanism of resistance to pazopanib. Thus, monitoring CAFs levels during treatment could have the potential to predict resistance and individualize treatment strategies. These findings warrant further investigation in larger clinical trials.

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Figures.

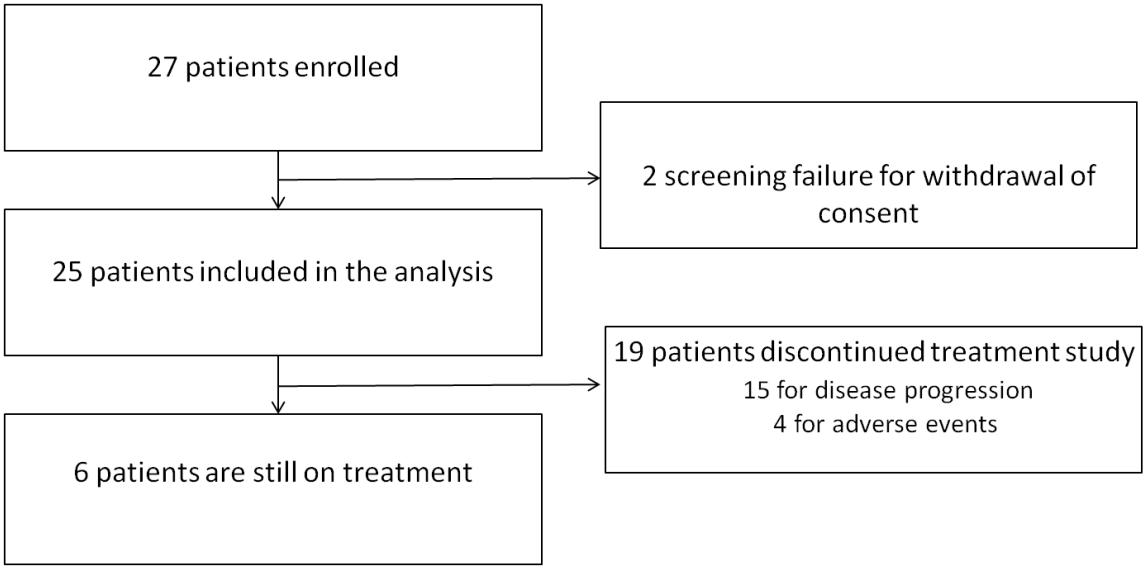


Fig 1. Treatment and follow up of the patients

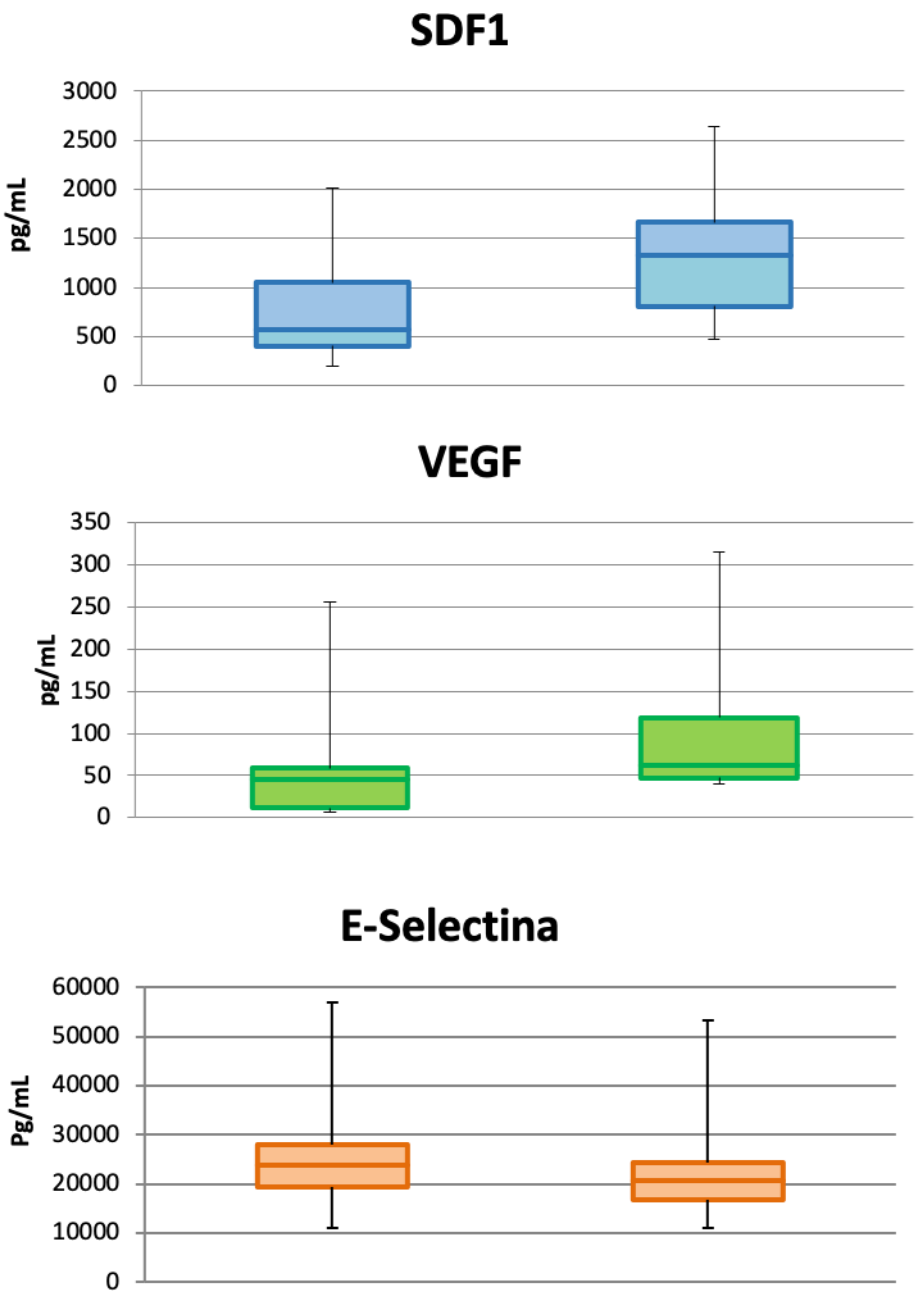


Fig 2. Significant changes in median CAF's plasma levels at PD compared to baseline during treatment with pazopanib

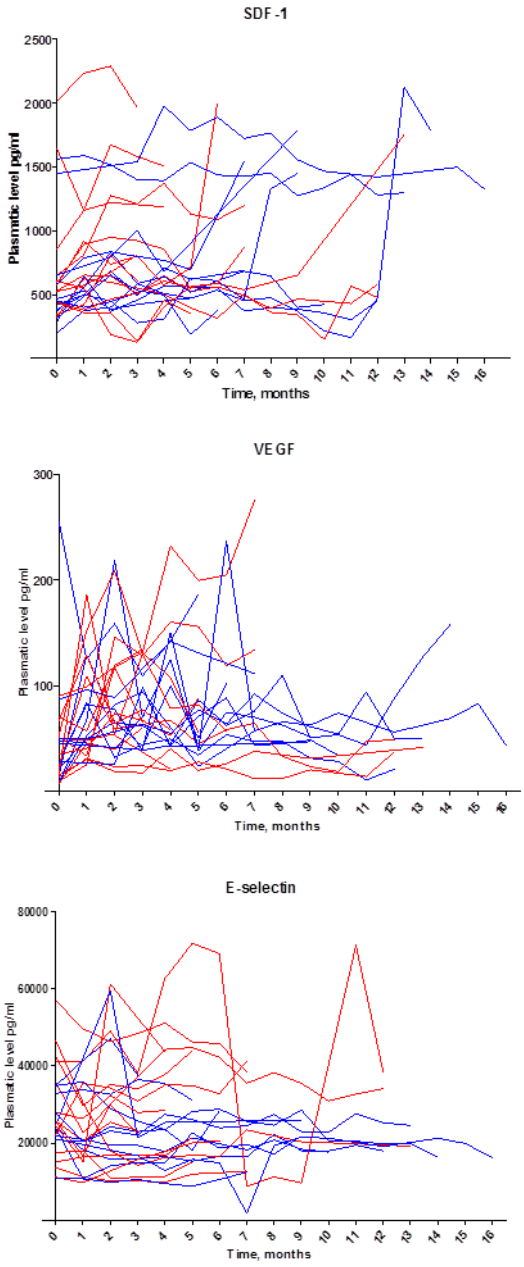


Fig 3. Change in CAF's level

In the Figure 3 the trend of the exact point values of CAFs (SDF-1, VEGF and E-selectin) at the different timepoints over time is graphically depicted. Blue lines: patients who achieved an objective response to treatment with pazopanib; Red lines: patients who did not achieve an objective response to treatment with pazopanib.

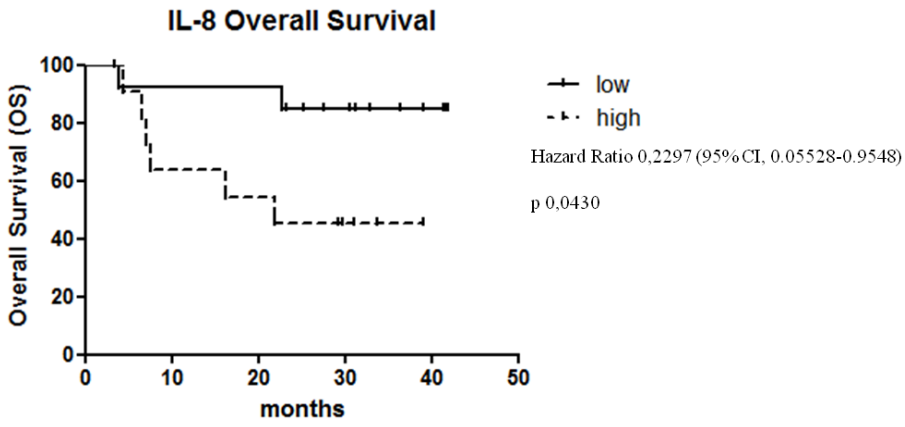


Fig 4. Kaplan-Meier Survival Curves of patients with low or high IL-8 levels at baseline

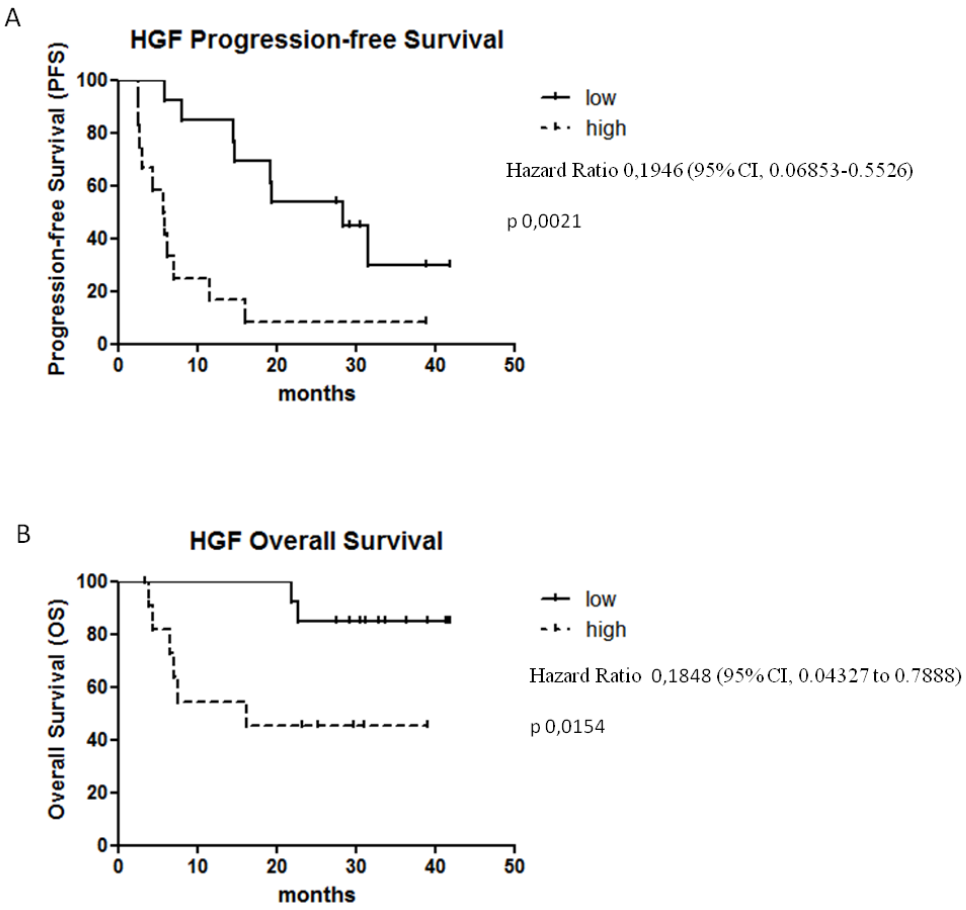
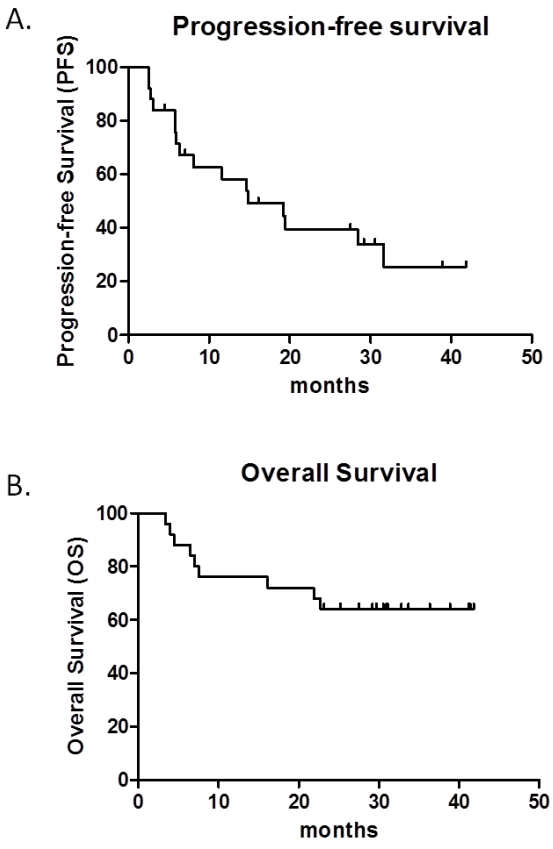
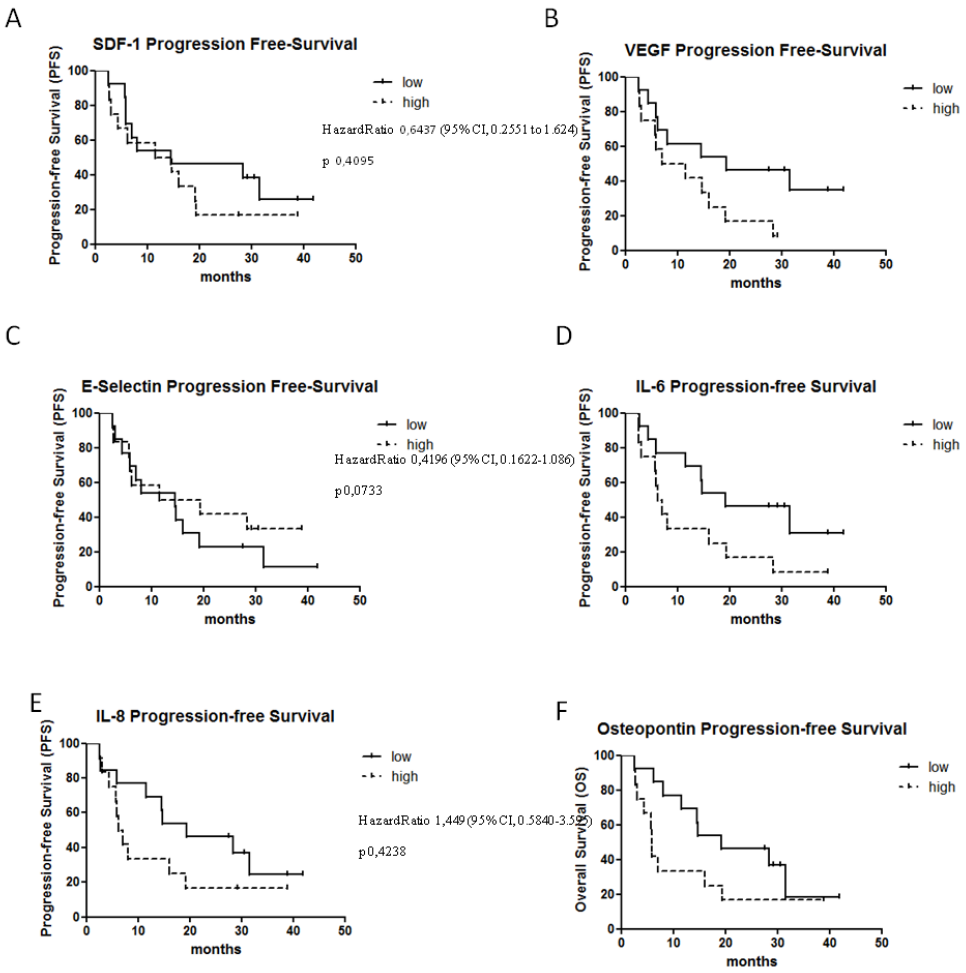


Fig 5. Progression-free survival (A) and overall survival (B) Kaplan-Meier Curves of patients with low or high HGF levels at baseline

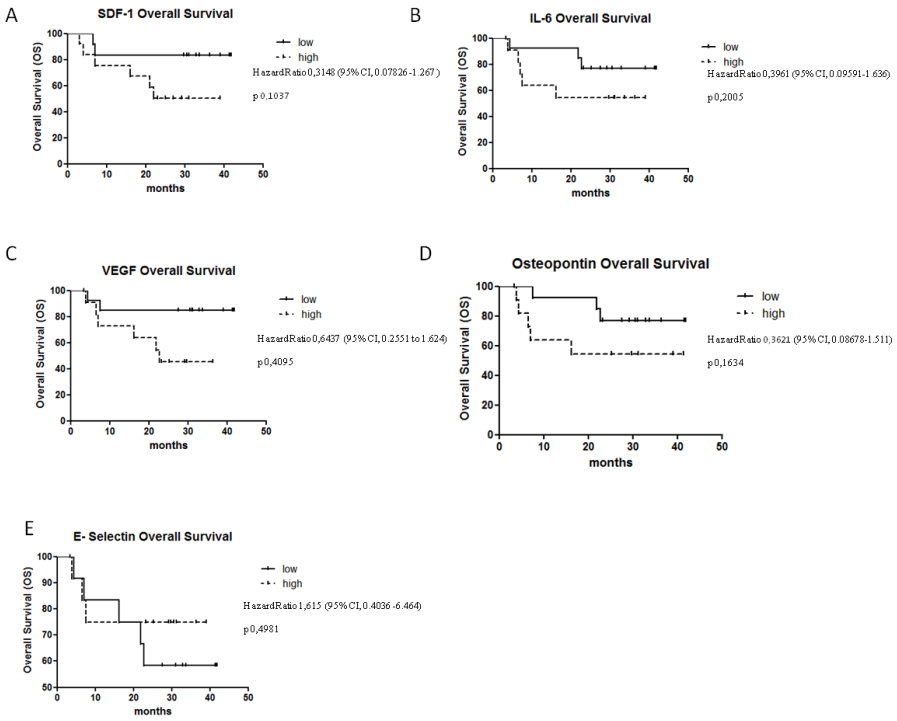


Suppl. Fig. 1. A) Progression free survival; B) Overall Survival



Suppl. Fig. 2 Progression-free survival Kaplan-Meier Curves of patients with low or high CAFs levels at baseline

(A) SDF-1. (B) IL-6. (C) VEGF. (D) IL-8. (E) E-selectin. (F) osteopontin.



Suppl. Fig. 3 Kaplan-Meier Survival Curves of patients with low or high CAFs levels at baseline

(A) SDF-1. (B) IL-6. (C) VEGF. (D) Osteopontin (E) E-selectin.

Tables.

Table 1. Baseline characteristics

Characteristic	No (%)
Age, years	65
Median (Range)	(54 – 73)
Sex	
Male	21 (84)
female	4 (16)
ECOG PS	
0	19 (76)
1	6 (24)
IMDC risk group	
Good	8 (32)
Intermediate	17 (68)
Prior Nephrectomy	
Yes	18 (72)
No	7 (28)
Site of metastases	
Lung	17 (68)
Bone	3 (12)
Lymphnodes	5 (20)
Other	12 (48)

Abbreviations: ECOG, Eastern operative group; PS, Performance Status IMDC, International Metastatic renal cell carcinoma database Consortium

Table 2. Association of baseline CAFs level with ORR to pazopanib

CAFs Low or high	Total	PR (%)	SD/PD (%)	Association with ORR in high versus low P value*
SDF1				0,238
low	13	8 (62)	5 (38)	
high	12	4 (33)	8 (67)	
VEGF				0,695
low	13	7 (54)	6 (46)	
high	12	5 (42)	7 (58)	
E-selectin				0,238
low	13	10 (62)	3 (38)	
high	12	2 (33)	10 (67)	
IL-6				**0,047**
low	13	9 (69)	4 (31)	
high	12	3 (25)	9 (75)	
IL -8				**0,047**
low	13	9 (69)	4 (31)	
high	12	3 (25)	9 (75)	
HGF				**0,005**
low	13	10 (77)	3 (23)	
high	12	2 (17)	10 (83)	
Osteopontin				**0,005**
low	13	10 (77)	3 (23)	
high	12	2 (17)	10 (83)	

Abbreviations: CAF's, circulating angiogenic factors: ORR, objective response rate; PR, partial response; SD, stable disease; PD, progressive disease

*P value according to Fisher Exact Test

Table 3. Association of baseline CAFs level with ORR to pazopanib

CAFs	Cutoff value (median baseline value)	Association with ORR in high versus low P value*
SDF1 (pg/mL)	525,93	0,238
VEGF-A (pg/mL)	27,80	0,695
E-selectin (pg/mL)	24.753,21	0,238
IL-6 (pg/mL)	5,69	**0,047**
IL -8 (pg/mL)	8,89	**0,047**
HGF (pg/mL)	71,62	**0,005**
Osteopontin (pg/mL)	53.484,39	**0,005**

Abbreviations: CAFs, circulating angiogenic factors; ORR, objective response rate

*P value according to Fisher Exact Test

Table 4. Change in CAFs plasma levels

CAFs	Median baseline value (range)	Median Value at PD (range)	P value
SDF1 (pg/mL)	574,67 (200,8-2.018,39)	1.328,03 (472,55-2.126,96)	**p = 0,011**
VEGF (pg/mL)	45,10 (6,16-256,14)	62,4 (39,42-186,74)	**p = 0,011**
E-selectin (pg/mL)	23.882,51 (11.016,44-56.948,61)	20.588,30 (10.991,75-38.415,71)	**p = 0,017**
IL-6 (pg/mL)	5,84 (0,63-261,34)	11,85 (0.10-70,33)	p = 0,57
IL -8 (pg/mL)	8,38 (1,52-38,769)	5,99 (0,89-25,89)	p = 0,61
HGF (pg/mL)	76,13 (27,85-259,65)	99,09 (39,8-241,89)	p = 0,39
Osteopontin (pg/mL)	53.484,39 (3.799,33-358.630,14)	77.641,32 (14.785,19-180.431,54)	p = 0,78

Abbreviations: CAFs, circulating angiogenic factors; PD, progressive disease

*P value according to Wilcoxon Test